**Preliminary Report**

**Familial relapsing haemolytic uraemic syndrome and complement factor H deficiency**


1Departments of Medicine and Human Genetics, University of Newcastle upon Tyne, Newcastle, 2Department of Pathology, University of Birmingham, 3Regional Immunology Department, Birmingham Heartlands Hospital, 4Department of Nephrology, The Birmingham Children’s Hospital, Birmingham, UK

**Abstract**

**Background.** In a recent study of three families we have found that inherited haemolytic uraemic syndrome (HUS) maps to a region of chromosome 1q containing the gene for complement factor H. In one of these families and also in a case of sporadic D-HUS, we have identified mutations in the factor H gene. A further family with inherited HUS has therefore been investigated.

**Methods.** DNA extracted from the family members and DNA extracted from archival post-mortem material from a deceased family member, was studied. Review of renal biopsies and study of complement components was also undertaken.

**Results.** This family demonstrates an inherited deficiency of complement factor H. Non-diarrhoeal HUS has affected at least two family members with half normal levels of factor H.

**Conclusion.** These findings represent further evidence of the association between factor H dysfunction and HUS.

**Key words:** complement; complement factor H; haemolytic uraemic syndrome

**Introduction**

Haemolytic uraemic syndrome (HUS) is characterized by the triad of Coombs test negative microangiopathic haemolytic anaemia, thrombocytopenia, and acute renal failure. It exists in a diarrhoea-associated, often epidemic form (D + HUS) typically caused by verocytotoxin producing bacteria such as *Escherichia coli* 0157:H7, as well as non-diarrhoeal forms (D-HUS) which are most often sporadic, relapsing or familial.

Two types of familial HUS are recognized [1]. In the first, affected individuals, particularly siblings usually residing in regions geographically related to outbreaks of HUS, suffer disease within days or weeks of each other and temporally related to the outbreak. It is likely that an infectious agent is responsible. The second familial form of HUS occurs at different times implying an inherited predisposition. In this form prognosis is poor, mortality is high (often over 50%) and recurrence is not uncommon. In a recent study of three families, we have found that inherited HUS maps to a region of chromosome 1q containing the gene for complement factor H. In one of these families, and also in a case of sporadic D-HUS, we have identified mutations in the factor H gene [2].

We now present a further family in which an inherited deficiency of complement factor H is associated with HUS.

**Family pedigrees and case histories (Figure 1)**

**II-4 (Index case)**

A previously well 5-month-old boy presented to his local hospital with a 2-week history of pallor and vomiting. There had been no diarrhoea. On examination he was found to be anaemic (Hb 5.6 g/dl) and hypertensive (systolic blood pressure between 120 and 140 mmHg). Urinalysis revealed microscopic haematuria and heavy proteinuria. Blood film showed burr cells and schistocytes. Over the next 2 weeks his platelet count fell from 245 to 52 × 10^9/l and his plasma creatinine rose from 58 to 95 μg/l.

A diagnosis of non-diarrhoeal haemolytic uraemic (D-HUS) was made and his care transferred to the Birmingham Children’s Hospital. His initial management consisted of transfusion with packed red cells, treatment of hypertension with labetalol and captopril and subsequently four whole blood exchanges over 2 weeks. A renal biopsy performed during this period showed collapsed glomeruli with widespread doubling of the glomerular capillary basement membranes and...
Fig. 1. Family pedigree, with corresponding complement profiles (normal ranges in parentheses) and genotypes. The gene for complement factor H has previously been mapped to chromosome 1q, between D1S212 and D1S306 [2]. A common haplotype bounded by the rectangle is therefore presumed to contain the faulty gene which results in haploinsufficiency of factor H. Complement studies were performed concurrently, during good health and in the absence of active HUS.

There was no chronic tubular damage and small arteries and arterioles appeared normal.

There was an initial improvement in his general condition, plasma creatinine (95–65 μg/l) and proteinuria (protein/creatinine ratio falling from 394 to 23 g/mol (N.R <20)). However 1 week later the blood film yet again revealed evidence of microangiopathy. This was treated with weekly whole blood exchanges using a right internal jugular (Broviac) catheter, and subsequently plasma exchanges using 4–600 ml of fresh frozen plasma (FFP).

A further relapse, characterized by anaemia, thrombocytopenia, heavy proteinuria and a rise in plasma creatinine to 100 μg/l, occurred at the age of 11 months after a respiratory viral infection. This was successfully treated with plasma exchange, and was followed by infusions of FFP on a weekly basis (10 ml/kg) as prophylaxis against further relapse.

This protocol was unsuccessful in preventing a third relapse, which proved refractory to daily infusions of FFP but responded to exchange transfusion. He was therefore treated with fortnightly exchange transfusions until the age of 24 months, when a twin lumen catheter was inserted and he received fortnightly plasma exchange with a PF1000 Gambro filter (1.5–2× estimated plasma volume, using equal volumes of 4.5% human albumin and FFP, the latter being given at the end of each treatment).

A fourth relapse at the age of 2.5 years old proved
The parents are healthy, unrelated Kenyan Asians.

II-1

The first-born child, a boy, died abruptly at 6 weeks of age, having previously been well. Post-mortem studies, however, revealed him to have been anaemic and uraemic (urea 20.4 mmol/l) with histological evidence of haemolytic uraemic syndrome. Glomeruli had widespread doubling of capillary basement membranes, and swollen mesangial and endothelial cells, with thrombosis in many capillary loops (Figure 3). There was no chronic tubular damage. Arteries and arterioles appeared normal in the kidney. There was also evidence of fibrinoid necrosis of a medium sized artery in the epididymis and intra-alveolar haemorrhage in all lobes of the lungs.

II-2

No relevant medical history.

II-3

The third child was admitted as an emergency at 7 months of age with a short illness marked by pallor, partially resistant to treatment, resulting in an inexorable decline in renal function over 12 months to end-stage renal failure. During this time it was discovered that the child had contracted hepatitis C, and because of abnormal liver function tests underwent a liver biopsy. This showed moderate portal tract infiltration with lymphocytes and histiocytes, mild fibrosis and haemosiderosis.

At age 5 he developed erythema nodosum for several months.

At 5.5 years he underwent a cadaveric renal transplant (1,2,0 mismatch at HLA-A,B and DR loci respectively) covered by immunosuppression with corticosteroids and azathioprine. Unfortunately acute cellular rejection occurred (proven on biopsy) and the graft was lost on day 11 following cortical rupture. Histological examination did not reveal any evidence of recurrent HUS.

Measurements of the plasma component of complement C3 have been consistently abnormal ranging from 0.27 to 0.72 g/l (mostly between 0.3 and 0.4). C1q and C4 were normal. Antinuclear antibodies were negative. Protein S, protein C and anti-thrombin III concentrations were normal. In infancy most measurements of von Willebrand antigen were elevated, although temporary depression of levels was seen post-plasma exchange (in a series of seven plasma exchanges, mean pre-exchange level was 1.73 U/ml (N.R. 0.5–1.5) and mean post-level was 1.23).

Plasma haptoglobin concentration was frequently subnormal, not only in the first year of life, but also subsequently when well on dialysis without other evidence of haemolysis.

The child is currently well on haemodialysis.
binding capacity 70.4 μmol/l) suggested iron deficiency. Three faecal occult blood tests were positive. Serum urea was 7.5 mmol/l and urine culture was negative (urinalysis not recorded).

He was managed by blood transfusion which corrected his anaemia and was discharged 5 days later on oral ferrous sulphate.

Six weeks later he was readmitted with a 2-day history of respiratory infection, diarrhoea and further anaemia (Hb 7.8 g/dl) with a microcytic blood film. A barium swallow showed gastro-oesophageal reflux and it was assumed that the iron deficiency reflected blood loss from the oesophagus, so he was treated with oral gaviscon (R + C) and oral iron.

He is now 17 years of age, of normal height and weight and has had no intervening illness. There is no evidence of current ill health, hypertension, renal impairment or HUS.

**Methods**

The study was approved by the Joint Ethics Committee, University of Newcastle upon Tyne and Newcastle and North Tyneside Health Authority. Following informed consent, blood was taken from surviving family members and permission was obtained to extract DNA from archival tissues.

**Genotyping**

DNA extraction and microsatellite polymorphism genotyping was performed as previously described [2].

**Complement studies**

Functional CH50 levels were measured by the method of Mayer [3], and expressed in units/ml. Functional alternative pathway activity was measured in agarose plates, using 0.5% guinea pig erythrocytes suspended in 1% agarose made with veronal buffered saline, pH 7.4, 0.01 M EGTA and 0.005 M MgCl2. Results were expressed as a percentage of pooled normal human plasma.

Factors H, B and P were measured immunochemically by single radial diffusion, using monospecific antisera (Binding site Ltd), results being expressed as a proportion of pooled normal plasma. C3 and C4 were measured by nephelometry (Behring) using standard human serum with assigned values (SPS 01, Sheffield Protein Reference Unit). C3 breakdown products were detected by immunoelectrophoresis of fresh EDTA plasma, using monospecific anti-C3d antiserum (Netherlands Red Cross BTS).

**Discussion**

The pathological findings in diarrhoeal and non-diarrhoeal HUS are significantly different. In D+HUS glomeruli have thrombosis but do not have basement membrane duplication [4], which was a prominent feature in the children we report. Intimal proliferation in the afferent arterioles, which is recognized in some patients with D-HUS was not seen in these cases.

Although it is now accepted that most cases of diarrhoeal HUS (D+HUS) are caused by verotoxin producing bacteria such as E. coli 0157:H7, the cause of non-diarrhoeal HUS has historically remained elusive. Previous theories have involved abnormalities of prostacyclin metabolism or of the amount and structure of von Willebrand factor.

We have recently postulated a theory of microangiopathy based on abnormalities of complement control molecules and in particular have implicated complement factor H, an important plasma bound regulator of the alternative pathway, in the aetiology of D-HUS [5]. This association was first described by Thompson and Winterborn [6]. They reported an 8-month-old boy who presented HUS with 5–10% normal levels of factor H, inherited in an autosomal recessive fashion from consanguineous parents, each of whom had approximately 50% normal levels. Since then two further families with inherited deficiencies of factor H have been described, from which one and two family members respectively, have suffered HUS [7,8]. Recently we have demonstrated that in three families HUS maps to a region of chromosome 1q containing the gene for complement factor H. In one of these families and also in a case of sporadic D-HUS we have identified mutations in the factor H gene [2].

Two recently published elegant papers have also implicated factor H deficiency in HUS. Ohali et al. [9] describe a large consanguineous Bedouin kindred with an autosomal recessive deficiency of factor H, in whom 10 infants have suffered D-HUS (and eight died). Of four affected individuals, all were hypocomplementaemic for C3 and deficient in factor H (with levels of factor of 0, 0, 25 and 40% of normal control sera, respectively). Rougier et al. [10] present six cases of factor H deficiency of whom five (three individuals and two cousins) suffered D-HUS. Two (the cousins) had less than 5% normal factor H levels and an absence of factor H upon immunoblotting, whereas the other three had approximately 50% levels (30, 40 and 45% respectively).

The family we describe in this paper includes at least two individuals who have suffered HUS. The index case was affected by relapsing HUS terminating upon the advent of end-stage renal failure. He demonstrates evidence of activation of the alternative pathway with very low levels of C3 and factor B in the presence of normal levels of C4. This is in the context of approximately half normal (35%) levels of complement factor H. His C3 nephritic factor has been consistently negative. In the absence of clinical manifestations his overall functional CH50 and alternative pathway haemolytic activity is normal, although during exacerbations of his illness alternative pathway haemolytic activity was suppressed.

The first child to have suffered HUS in this family died at the age of 6 weeks with a retrospective diagnosis of HUS. As this child has an identical genotype over the region of chromosome 1 previously shown to contain the gene for factor H, we have assumed that he also would have been haplodeficient for serum factor H.
A third child also has half normal levels of factor H, however with a less pronounced fall in C3 and factor B levels. He suffered a relapsing illness early in life characterized by anaemia and a slightly raised serum urea, however we cannot be sure that this represents HUS and could have been caused by upper gastrointestinal blood loss. It may be relevant that although he carries the presumed faulty gene that causes half normal levels of factor H (boxed in Figure 1), he has inherited a different paternal gene than the affected children II-1 and II-4. Could it be that these affected individuals carry one faulty gene which causes half normal factor H levels and one faulty gene that causes malfunction rather than deficiency of factor H—i.e. reflecting an autosomal recessive disorder with two different gene mutations? Or it may be that there is another genetic defect also predisposing to HUS, which when combined with half normal levels of factor H results in the more severe clinical form. The mother of the family from whom the ‘haplodeficiency’ gene seems to have been inherited, demonstrates a remarkably normal complement profile.

An alternative theory is that haplodeficiency for factor H is sufficient in itself to cause HUS. It is notable that four of the recently published cases of HUS had approximately 50% of normal levels of factor H [9,10]. Precedent exists elsewhere within the complement pathway. Haplodeficiency for C1 esterase inhibitor predisposes to angioneurotic oedema. One could postulate a ‘dose effect’ which becomes important following an environmental trigger. Perhaps an infection at an early age precipitates a state of clinical complement activity, which the low levels of factor H are unable to sufficiently damp down.

It is also possible that the many recognized polymorphisms of the genes coding for other complement control molecules play a role in defining phenotype.

Two types of gene mutation have been described in humans with factor H deficiency:

(i) We have previously identified a 4 base-pair deletion in exon 1 of the gene, which causes a frameshift and subsequent premature stop codon, found in an individual with relapsing HUS and half normal levels of factor H [2].

(ii) The genetic and cellular basis of hypocomplementaemic mesangiocapillary glomerulonephritis in a factor H deficient child has also recently been reported. Using the techniques of immunofluorescent staining and confocal microscopic imaging of cultured fibroblasts, it was demonstrated that factor H secretion rather than the production was impaired. Two mutations affecting cysteine residues on each allele were shown to be responsible for this deficiency [11].

Genetic studies have so far been unsuccessful in identifying the gene mutations in the family we have described. They do not appear to have either of the above two mutations.

References

7. Roodhooff AM, McLean RH, Elst E, Van Acker KJ. Recurrent infection at an early age precipitates a state of clinical complement activity, which the low levels of factor H are unable to sufficiently damp down.

Received for publication: 22.10.98
Accepted in revised form: 18.1.99